

CLAIMS

What is claimed is:

1. An isolated nucleic acid encoding a mammalian Lhx3, wherein said nucleic acid is selected from the group consisting of an isolated nucleic having at least about 88% identity with at least one of SEQ ID NO:1, SEQ ID NO:13, and SEQ ID NO:15, and further wherein said isolated nucleic acid has at least about 88.5% identity with at least one of SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:11.

2. The isolated nucleic acid of claim 1, wherein the nucleic acid sequence of said nucleic acid is SEQ ID NO:1.

3. An isolated nucleic acid encoding a porcine Lhx3, said nucleic acid having at least about 88% identity with a nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:13, and SEQ ID NO:15.

4. An isolated nucleic acid encoding a human Lhx3, said nucleic acid having at least about 88.5% identity with a nucleic acid having the nucleotide sequence of at least one of SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:11.

5. The isolated nucleic acid of claim 1, said nucleic acid further comprising a nucleic acid encoding a tag polypeptide covalently linked thereto.

6. The isolated nucleic acid of claim 5, wherein said tag polypeptide is selected from the group consisting of a myc tag polypeptide, a glutathione-S-transferase tag polypeptide, a green fluorescent protein tag polypeptide, a myc-pyruvate kinase tag polypeptide, a His6 tag polypeptide, an influenza virus hemagglutinin tag polypeptide, and a maltose binding protein tag polypeptide.

7. The isolated nucleic acid of claim 1, said nucleic acid further comprising a nucleic acid encoding a promoter/regulatory sequence operably linked thereto.
8. A vector comprising the nucleic acid of claim 1.
9. A recombinant cell comprising the isolated nucleic acid of claim 1.
10. An isolated polypeptide encoded by the nucleic acid of claim 1.
11. The isolated polypeptide of claim 1, wherein said polypeptide shares at least about 93% identity with a polypeptide having the amino acid sequence SEQ ID NO:2.
12. The isolated polypeptide of claim 1, wherein said polypeptide shares at least about 94.5% identity with a polypeptide having the amino acid sequence of at least one of SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12.
13. An antibody that specifically binds with the isolated polypeptide of claim 10, or a fragment thereof.
14. The antibody of claim 13, wherein said antibody specifically binds with a portion of said isolated polypeptide selected from the group consisting of a portion from about amino acid residue 1 to about amino acid residue 26 of SEQ ID NO:10, a portion from about amino acid residue 1 to about amino acid residue 31 of SEQ ID NO:12, a portion from about amino acid residue 1 to about amino acid residue 29 of SEQ ID NO:14, and a portion from about amino acid residue 1 to about amino acid residue 31 of SEQ ID NO:16.

15. The antibody of claim 13, wherein said antibody is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, and a synthetic antibody.

16. A composition comprising the isolated nucleic acid of claim 1 and a pharmaceutically-acceptable carrier.

17. A composition comprising the isolated polypeptide of claim 10 and a pharmaceutically-acceptable carrier.

18. An isolated nucleic acid encoding a mammalian Lhx3, wherein said nucleic acid specifically hybridizes under high stringency conditions to a nucleic acid selected from the group consisting of a nucleic acid having the nucleic acid sequence SEQ ID NO:1, a nucleic acid having the nucleic acid sequence SEQ ID NO:7, a nucleic acid having the nucleic acid sequence SEQ ID NO:9, a nucleic acid having the nucleic acid sequence SEQ ID NO:11, a nucleic acid having the nucleic acid sequence SEQ ID NO:13, and a nucleic acid having the nucleic acid sequence SEQ ID NO:15, or a nucleic acid complementary to any one of SEQ ID NO:1, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, and SEQ ID NO:15.

19. An isolated nucleic acid having at least about 45% identity with from about nucleotide -104 to about nucleotide 78 of SEQ ID NO:9.

20. An isolated nucleic acid having at least about 67% identity with from about nucleotide -119 to about nucleotide 93 of SEQ ID NO:11.

21. A method for detecting the presence or absence of a nucleic acid encoding a mammalian Lhx3 having at least about 88.5% homology with a nucleic acid having the sequence SEQ ID NO:7 in a sample, said method comprising

(a) contacting a sample with a nucleic acid probe or primer which specifically hybridizes with said nucleic acid; and

(b) determining whether said nucleic acid probe or primer binds with a nucleic acid in said sample whereby when said nucleic acid probe or primer binds with a nucleic acid in said sample, said sample contains a nucleic acid which specifically hybridizes with said nucleic acid,

thereby detecting the presence or absence of said nucleic acid in a sample .

22. The method of claim 21, wherein said sample comprises mRNA molecules.

23. A method for detecting the presence or absence of a nucleic acid encoding a mammalian Lhx3 in a sample, said method comprising

(a) amplifying a nucleic acid encoding a mammalian Lhx3 present in a sample wherein said nucleic acid shares about 88.5% identity with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, and SEQ ID NO:15;

(b) detecting any amplified target nucleic acid formed in (a) whereby when said nucleic acid is amplified said sample contains said nucleic acid which encodes a mammalian Lhx3,

thereby detecting the presence or absence of a nucleic acid encoding a mammalian Lhx3 in a sample.

24. A method of quantitatively detecting a nucleic acid encoding a mammalian Lhx3 in a sample, said method comprising

(a) contacting a target nucleic acid encoding a mammalian Lhx3 present in a sample with polymerase chain reaction reagents specific for said target nucleic acid, said polymerase chain reaction reagents including at least two polymerase

chain reaction primers, a polymerase enzyme, and an internal fluorescent oligonucleotide probe, said oligonucleotide probe further comprising:

(i) a sequence capable of hybridizing to a portion of said target nucleic acid;

(ii) a fluorescer molecule attached to the 3' end of the oligonucleotide;

(iii) a quencher molecule attached to the 5' end of said oligonucleotide capable of substantially quenching said fluorescer molecule when said oligonucleotide is intact, wherein said fluorescer molecule becomes substantially unquenched when said oligonucleotide probe is cleaved by DNA polymerase during amplification of the target nucleic acid; and

(iv) said 3' end is impervious to the 5'→3' extension activity of said polymerase enzyme; and

(b) amplifying the target nucleic acid by thermal cycling, wherein said thermal cycling is sufficient to amplify said target nucleic acid; and

(c) measuring the level of fluorescence in said sample subsequent to thermal cycling, and further wherein the level of fluorescence is correlated to the amount of target nucleic acid present in the sample, thereby quantitatively detecting a target nucleic acid encoding a mammalian Lhx3 in a sample.

25. The method of claim 24, wherein said polymerase chain reaction primer is selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:44, and a nucleic acid having the sequence of SEQ ID NO:45.

26. A kit comprising a compound which specifically hybridizes with a nucleic acid having at least about 88.5% homology to a nucleic acid having the sequence of at least one of SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:11, and an instructional material for the use thereof.

27. The kit of claim 26, wherein said compound is selected from the group consisting of a nucleic acid and an antibody.

28. The kit of claim 27, wherein said nucleic acid is selected from the group consisting of a polymerase chain reaction primer, and an internal oligonucleotide probe.

29. A kit for detecting a nucleic acid encoding a mammalian Lhx3, said kit comprising a first nucleic acid having the sequence selected from the group consisting of SEQ ID NO:34, and SEQ ID NO:36, and a second nucleic acid having the sequence selected from the group consisting of SEQ ID NO:35, and SEQ ID NO:37, and an instructional material for the use thereof.

30. The kit of claim 29, further comprising an internal oligonucleotide probe complementary to at least a portion of a nucleic acid having the sequence of from about nucleotide 1 to about nucleotide 8867 of SEQ ID NO:22.

31. The kit of claim 30, wherein said oligonucleotide probe has the sequence selected from the group consisting of SEQ ID NO:38, SEQ ID NO:39, and SEQ ID NO:40.

32. A method of quantitatively detecting a nucleic acid encoding a mammalian Lhx3a in a sample, said method comprising

(a) contacting a target nucleic acid encoding a mammalian Lhx3a present in a sample with polymerase chain reaction reagents specific for said target nucleic acid, said polymerase chain reaction reagents including at least two polymerase chain reaction primers, a polymerase enzyme, and an internal fluorescent oligonucleotide probe, the oligonucleotide probe further comprising:

(i) a sequence capable of hybridizing to a portion of said target nucleic acid wherein said portion is unique to Lhx3a;

- (ii) a fluorescer molecule attached to a 5' end of said oligonucleotide;
- (iii) a quencher molecule attached to a 3' end of said oligonucleotide capable of substantially quenching said fluorescer molecule when said oligonucleotide is intact, wherein said fluorescer molecule becomes substantially unquenched when said oligonucleotide probe is cleaved by DNA polymerase during amplification of the target nucleic acid; and
- (iv) a 3' end which is impervious to the 5'→3' extension activity of the DNA polymerase; and

(b) amplifying the target nucleic acid by thermal cycling, wherein said thermal cycling is sufficient to amplify said target nucleic acid; and

(c) measuring the level of fluorescence in said sample subsequent to thermal cycling, and further wherein the level of fluorescence is correlated to the amount of target nucleic acid present in the sample, thereby quantitatively detecting a target nucleic acid encoding a mammalian Lhx3a in a sample.

33. The method of claim 32, wherein said polymerase chain reaction primer is selected from the group consisting of a first nucleic acid having a sequence selected from the group consisting of SEQ ID NO:34, and a second nucleic acid having a sequence selected from the group consisting of SEQ ID NO:35.

34. A kit for quantitatively detecting a nucleic acid encoding a mammalian Lhx3a, said kit comprising a polymerase chain reaction primer having the sequence selected from the group consisting of SEQ ID NO:34, and SEQ ID NO:35, and an instructional material for the use thereof.

35. The kit of claim 34, further comprising an internal oligonucleotide probe which specifically hybridizes with a nucleic acid molecule comprising a nucleic acid having the sequence of SEQ ID NO:38.

36. The kit of claim 35, wherein said internal oligonucleotide probe has the sequence SEQ ID NO:40.

37. A kit for quantitatively detecting a nucleic acid encoding a mammalian Lhx3b, said kit comprising a polymerase chain reaction primer having the sequence selected from the group consisting of SEQ ID NO:36, and SEQ ID NO:37, and an instructional material for the use thereof.

38. The kit of claim 37, further comprising an internal oligonucleotide probe which specifically hybridizes with a nucleic acid molecule having the sequence of SEQ ID NO:39.

39. A method of quantifying the level of Lhx3 expressed in a cell, the method comprising, reverse transcribing any ribonucleic acid encoding Lhx3 present in a cell, amplifying any deoxyribonucleic acid encoding Lhx3 produced by reverse transcription, and detecting any amplified deoxyribonucleic acid formed using quantitative sequence detection, thereby quantifying the level of Lhx3 expressed in a cell.

40. The method of claim 39, wherein said Lhx3 is selected from the group consisting of porcine Lhx3a, porcine Lhx3b, human Lhx3a, and human Lhx3b.

41. The method of claim 39, wherein said amplification comprises amplification using polymerase chain reaction wherein said polymerase chain reaction comprises a primer having a sequence selected from the group consisting of SEQ ID NO:41, and SEQ ID NO:35.

42. The method of claim 39, wherein said detection comprises contacting any deoxyribonucleic acid produced with an internal fluorescent oligonucleotide probe which specifically hybridizes with a nucleic acid of claim 1.

43. A kit for detecting a nucleic acid encoding a mammalian Lhx3a, said kit comprising a first nucleic acid having the sequence SEQ ID NO:34, and a second nucleic acid having the sequence SEQ ID NO:35, and an instructional material for the use thereof.

44. The kit of claim 43, further comprising an internal oligonucleotide probe complementary to at least a portion of a nucleic acid having the sequence of SEQ ID NO:9.

45. A kit for detecting a nucleic acid encoding a mammalian Lhx3b, said kit comprising a first nucleic acid having the sequence SEQ ID NO:36, and a second nucleic acid having the sequence SEQ ID NO:37, and an instructional material for the use thereof.

46. The kit of claim 45, further comprising an internal oligonucleotide probe complementary to at least a portion of a nucleic acid having the sequence of SEQ ID NO:11.

47. The kit of claim 46, wherein the oligonucleotide probe has the sequence SEQ ID NO:40.

48. A method of identifying a compound that affects expression of human Lhx3 in a cell, said method comprising contacting a cell with a compound and comparing the level of expression of Lhx3 in said cell contacted with said compound with the level of expression of Lhx3 in an otherwise identical cell, wherein a higher or lower level of expression of Lhx3 in said cell contacted with said compound compared with the level of expression of Lhx3 in said otherwise identical cell not contacted with said compound, is an indication that said compound affects expression of Lhx3 in a cell.

49. The method of claim 48, wherein said human Lhx3 is selected from the group consisting of hLhx3a, and hLhx3b.

50. A method of identifying a compound that affects the level of expression of Lhx3a but not the level of expression of Lhx3b in a cell, said method comprising contacting a cell with a compound and comparing the level of expression of Lhx3a in a cell with the level of expression of Lhx3a in an otherwise identical cell not contacted with said compound, and further comparing the level of expression of Lhx3b in said cell contacted with said compound with the level of expression of Lhx3b in said otherwise identical not contacted with said compound, wherein a higher or lower level of expression of Lhx3a in said cell contacted with said compound compared with the level of expression of Lhx3a in said otherwise identical cell not contacted with said compound, and further wherein there is no detectable change in the level of expression of Lhx3b in said cell contacted with said compound compared to the level of expression of Lhx3b in said otherwise identical cell not contacted with said compound, is an indication that said compound affects the level of expression of Lhx3a but not the level of expression of Lhx3b in a cell.

51. A method of identifying a compound that affects the level of expression of Lhx3b but not the level of expression of Lhx3a in a cell, said method comprising contacting a cell with a compound and comparing the level of expression of Lhx3b in a cell with the level of expression of Lhx3b in an otherwise identical cell not contacted with said compound, and further comparing the level of expression of Lhx3a in said cell contacted with said compound with the level of expression of Lhx3a in said otherwise identical not contacted with said compound, wherein a higher or lower level of expression of Lhx3b in said cell contacted with said compound compared with the level of expression of Lhx3b in said otherwise identical cell not contacted with said compound, and further wherein there is no detectable change in the level of expression of Lhx3a in said cell contacted with said compound compared to the level of

expression of Lhx3a in said otherwise identical cell not contacted with said compound, is an indication that said compound affects the level of expression of Lhx3b but not the level of expression of Lhx3a in a cell.

52. A method of identifying a compound which affects the activity of human Lhx3 in a cell, said method comprising contacting a cell with a compound and comparing the level of activity of Lhx3 in said cell contacted with said compound with the level of activity of Lhx3 in an otherwise identical cell not contacted with said compound, wherein a higher or lower level of activity of Lhx3 in said cell contacted with said compound compared with the level of activity of Lhx3 in said otherwise identical cell not contacted with said compound, is an indication that said compound affects the activity of Lhx3 in a cell.

53. The method of claim 52, wherein said activity of said human Lhx3 is selected from the group consisting of the ability of hLhx3 to induce expression of a reporter gene operably linked to a α GSU promoter, the ability of hLhx3 to induce expression of a reporter gene operably linked to a TSH β promoter, the ability of hLhx3 to bind to the -350 to -323 bp region of murine α GSU promoter, the ability to activate the gene encoding Pit-1, and the ability to activate the gene encoding prolactin.

54. The method of claim 52, wherein said human Lhx3 is selected from the group consisting of Lhx3a, and Lhx3b.

55. A method of identifying a compound which affects the level of activity of Lhx3 in a cell, said method comprising

- (a) transfecting a cell with a vector encoding Lhx3;
- (b) co-transfecting said cell transfected with a reporter gene construct wherein said construct comprises a reporter gene operably linked to a promoter/regulatory sequence that is *trans*-activated by Lhx3;
- (c) contacting said cell of (b) with a test compound;

(d) measuring the level of activity of Lhx3 in said cell of (b) before and after contacting said cell with said compound;

(f) comparing the level of activity of Lhx3 in said cell contacted with said compound with the level of activity of Lhx3 in said cell prior to or in the absence of contacting said cell with said compound;

wherein a higher or lower level of activity of Lhx3 in said cell contacted with said compound compared with the level of activity of Lhx3 in said cell prior to or in the absence of contacting said cell with said compound is an indication that said compound affects the level of activity of Lhx3 in a cell.

56) A method of identifying a compound which affects the level of activity of Lhx3a but not the level of activity of Lhx3b in a cell, said method comprising

(a) transfecting a cell having no detectable endogenous Lhx3a activity with an expression vector encoding Lhx3a;

(b) transfecting an otherwise identical cell said cell having no detectable endogenous Lhx3b activity with an expression vector encoding Lhx3b;

(c) co-transfecting each of said cells in (a) and (b) with a reporter gene construct comprising a reporter gene operably linked to a promoter/regulatory sequence *trans*-activated by Lhx3a and Lhx3b;

(d) contacting each of said cells of (c) with a test compound;

(e) measuring the level of Lhx3a activity in each of said cells of (c) before and after contacting each of said cells with said test compound;

(f) measuring the level of Lhx3b activity in each of said cells of (c) before and after contacting each of said cells with said test compound;

(g) comparing the level of activity of Lhx3a in each of said cells of (c) contacted with said compound to the level of activity of Lhx3a in each of said cells prior to or in the absence of contacting each of said cells with said test compound;

(h) comparing the level of activity of Lhx3b in each of said cells of (c) contacted with said test compound to the level of activity of Lhx3b in each of said cells prior to or in the absence of contacting each of said cells with said test compound,

wherein a higher or lower level of activity Lhx3a in each of said cells of (c) contacted with said test compound compared to the level of activity of Lhx3a in each of said cells prior to or in the absence of contacting each of said cells with said test compound and further wherein there is no detectable change in the level of Lhx3b activity in each of said cells of (c) contacted with said test compound compared to the level of Lhx3b activity in each of said cells of (c) prior to or in the absence of contacting each of said cells with said test compound, is an indication that said test compound affects the level of Lhx3a activity but not the level of Lhx3b activity in a cell.

57. A method of identifying a compound which affects binding of Lhx3 to a nucleic acid that specifically binds with Lhx3, said method comprising administering a compound into an extract wherein said extract comprises Lhx3 and a nucleic acid that specifically binds with Lhx3, and comparing the level of binding of Lhx3 with said nucleic acid in said extract comprising said compound with the level of binding of Lhx3 with said nucleic acid in an otherwise identical extract which does not comprise said compound, wherein a higher or lower level of binding of Lhx3 with said nucleic acid in said extract comprising said compound compared to the level of binding of Lhx3 to said nucleic acid in said otherwise identical extract not comprising said compound, is an indication that said compound affects binding of Lhx3 with a nucleic acid that specifically binds with Lhx3.

58. The method of claim 57, wherein said Lhx3 is selected from the group consisting of human Lhx3a, human Lhx3b, porcine Lhx3a and porcine Lhx3b.

59. The method of claim 58, wherein said nucleic acid that specifically binds with Lhx3 is selected from the group consisting of a nucleic acid having

nucleotides -350 to -323 of murine α GSU promoter (SEQ ID NO:42), and a nucleic acid encoding the Lhx3 consensus binding sequence (SEQ ID NO:43).

60. A method of identifying a compound that affects Lhx3 induction of a pituitary trophic hormone gene promoter, said method comprising

- (a) transfecting a cell with a reporter gene construct wherein said construct comprises a thyroid-stimulating hormone beta promoter sequence operably linked to a reporter gene;
- (b) transfecting said cell of (a) with a hLhx3 expression vector;
- (c) contacting said cell of (b) with a pituitary transcription factor which synergizes with Lhx3;
- (d) contacting said cell of (c) with a compound;
- (e) assessing the level of expression of said reporter gene where the level of expression of said reporter gene is correlated to the level of Lhx3 induction of said promoter; and
- (f) comparing the level of expression of said reporter gene in said cell contacted with said compound with the level of expression of said reporter gene in an otherwise identical cell not contacted with said compound,

wherein in a higher or lower level of expression of said reporter gene in said cell contacted with said compound compared with the level of expression of said reporter gene in said otherwise identical cell not contacted with said compound, is an indication that said compound affects Lhx3 induction of a pituitary trophic hormone gene promoter in a cell.

61. The method of claim 60, wherein said pituitary trophic hormone gene promoter is selected from the group consisting of a thyroid-stimulating hormone beta promoter, an alpha-glycoprotein subunit promoter, and a prolactin promoter.

62. The method of claim 60, wherein said pituitary transcription factor which synergizes with Lhx3 is selected from the group consisting of Pit-1, Pitx1/P-Otx, and thyrotrope embryonic factor.

63. The method of claim 60, wherein said Lhx3 is selected from the group consisting of Lhx3a, Lhx3b, pLhx3a, and pLhx3b.

64. A method of identifying a human patient afflicted with a disease, disorder or condition associated with altered expression of Lhx3, said method comprising detecting the level of Lhx3 expression in a human and comparing said level of expression of Lhx3 in said human with the level of expression of Lhx3 in a normal human not afflicted with a disease, disorder or condition associated with altered expression of Lhx3, thereby detecting a human patient afflicted with a disease, disorder or condition associated with altered expression of Lhx3.

65. A method of identifying a human patient afflicted with a disease, disorder or condition associated with altered level of binding of Lhx3 to a nucleic acid that specifically binds with Lhx3, said method comprising detecting the level of Lhx3 binding with a nucleic acid that specifically binds with Lhx3 in a human and comparing said level of Lhx3 binding with a nucleic acid that specifically binds with Lhx3 in said human with the level of Lhx3 binding with a nucleic acid that specifically binds with Lhx3 in a normal human not afflicted with a disease, disorder or condition associated with altered level of Lhx3 binding with a nucleic acid that specifically binds with Lhx3, wherein a higher or lower level of Lhx3 binding with a nucleic acid that specifically binds with Lhx3 in said human compared with the level of Lhx3 binding with a nucleic acid that specifically binds with Lhx3 in said human not afflicted with a disease, disorder or condition associated with altered level of Lhx3 binding with a nucleic acid that specifically binds with Lhx3, is an indication that said human is afflicted with a disease, disorder or condition associated with altered level of Lhx3 binding with a nucleic acid that specifically binds with Lhx3, thereby detecting a

human patient afflicted with a disease, disorder or condition associated with altered level of Lhx3 binding with a nucleic acid that specifically binds with Lhx3.

66. The method of claim 65, wherein said nucleic acid that specifically binds with Lhx3 is selected from the group consisting of nucleotides -350 to -323 of murine α GSU promoter, and the Lhx3 consensus binding sequence.

67. A method of detecting a mutation in a Lhx3 allele in a human, said method comprising comparing the nucleic acid sequence encoding Lhx3 of a human suspected of having a mutation in Lhx3 with the nucleic acid sequence encoding Lhx3 obtained from a normal human not having a mutation in Lhx3, wherein any difference between said nucleic acid sequence of said human suspected of having a mutation in Lhx3 and said nucleic acid sequence encoding Lhx3 of said normal human not having a mutation in Lhx3 detects a mutation in a Lhx3 allele in said human.

68. A method of detecting a mutation in a Lhx3 allele in a human, said method comprising comparing the genomic nucleic acid sequence encoding Lhx3 of a human suspected of having a mutation in Lhx3 with the genomic nucleic acid sequence encoding Lhx3 obtained from a normal human not having a mutation in Lhx3, wherein any difference between said genomic nucleic acid sequence of said human suspected of having a mutation in Lhx3 and said genomic nucleic acid sequence encoding Lhx3 of said normal human not having a mutation in Lhx3 detects a mutation in a Lhx3 allele in said human.